**QUALITY CONTROL MATERIALS FOR GENETIC TESTING CONFERENCE**
**SEPT. 15-16, 2003, ATLANTA, GA**

## Summary

### Introduction

In the opening presentation, Dr. D. Joe Boone provided a brief outline of CDC efforts to address the needs for QC/PT materials for genetic tests and questions to be answered in establishing sustainable processes for development, collection, validation, and distribution of QC materials. Dr. Lawrence M. Silverman gave an overview on guidelines, oversight, and availability of current quality control materials for genetic tests. Specific quality control issues and challenges were highlighted for a number of testing areas and technology, including the CF panel testing, molecular HLA testing, in situ hybridization-based testing, molecular microbiology testing, sequencing-based diagnostics, and SNP genotyping after whole genome amplification.

### Current Research Efforts on Developing QC Materials

Stabilized Synthetic Nucleic Acids

Dr. Clark Rundell, Maine Molecular Quality Controls, Inc., presented his experience in generating synthetic control materials for CF and thrombotic risk testing using *in vitro* mutagenesis techniques. The process is intended to provide validated and stabilized DNA control constructs suitable for monitoring test performance from sample extraction through the entire analytical procedure.

Cell Transformation

Dr. Tim Stenzel, Visis, Inc., reported a CDC-funded effort to develop and pilot test a process for establishing and validating stably transformed cell lines as positive control materials for molecular genetic testing, using anonymous residual specimens from participating laboratories. This work has generated positive control materials for a number of genetic tests, including CF, Fragile X, hereditary hemochromatosis, factor V Leiden, and MTHFR. Several cell lines containing CF mutations for which control materials were previously unavailable have been stably established and validated. The control materials developed by this project are available for use in performance evaluation and quality assurance of genetic testing through Corriell, which serves as the repository and distributor of the cell lines.

Genetic Engineering

Dr. Wayne Grody presented another CDC-funded project to prepare and make available positive control materials for molecular genetic testing, by creating null cell lines using homologous recombination and introducing gene constructs containing target mutations into these cell lines to mimic natural mutation-containing human samples. Using this strategy, artificial cell lines have been created for the G85E and 1078delT mutations of the CFTR gene, which have been pilot tested using reverse ASO line blots, microarray, sequencing, ARMS, and other methodology. This project is currently ongoing with plans to generate control materials for additional CF mutations, BRCA1/2 mutations, trinucleotide repeat expansions, cancer markers, and infectious diseases.

Development and Use of QC Materials in European Countries

Dr. Elizabeth Dequeker reviewed the role of appropriate QC materials in validation and quality assurance of genetic testing and sources of QC materials in European countries. She pointed out that not including appropriate control materials was a major cause of genotyping errors in European External Quality Assessment (EQA) schemes for genetic testing. Dr. Barton provided an overview of the CRMGEN project, a European project to develop reference materials for genetic tests.

Dried Blood Spot Materials

Dr. Joanne Mei gave an overview of the dried blood spots-based quality assurance challenges provided by the CDC Newborn Screening Quality Assurance Program. She concluded that dried blood spots provided a stable matrix for both phenotypic and genotypic analyses, and CDC would continue to provide EQA challenges for newborn screening testing using dried blood spots.

Multiplex Control Materials

Dr. Roger Lebo, Children's Hospital Medical Center of Akron, OH, presented a process using PCR methodology to generate DNA sequences containing multiple mutations that can be used as control materials for CF and Huntington disease testing. The synthesized multiplex controls are intended to provide efficient quality control for panel testing and other multiplex assays.

Synthetic Standards for Gene Expression Analysis

Dr. James Willey, Medical College of Ohio, Toledo, OH, described an approach to generating standardized mixtures of cDNA internal standards for gene expression analysis using RT-PCR methodology. This approach is intended to allow standardized measurement of gene expression and comparison of results generated by different researchers.

Future Needs for QC Materials

Dr. Ira Lubin, Division of Laboratory Systems, Public Health Practice Program Office, CDC, summarized the participants' responses and comments on the greatest areas of needs for QC materials at present and within the next five years.

**Panel Discussion**

Panel Discussion on Current Research Efforts and Needs for QC Materials

The panel discussion was moderated by Dr. Toby Merlin. The panelists included Dr. Sue Richards, Dr. Tim Stenzel, Dr. Margaret McGovern, and Dr. Emily Winn-Deen.

*Discussion on Presented Approaches*

The panel discussed the strengths and weaknesses of the approaches to developing QC materials presented at the meeting and their applicability to various areas of genetic testing needing QC materials. The participants provided the following comments:

- Cell lines should be considered a preferred choice for quality control materials. However, it may be time-consuming to find and collect cell lines that contain the mutations needed for positive QC, and it is unlikely for any naturally occurring cell line to contain multiple

mutations in a single gene. Genetic engineering has the ability to introduce the mutations needed into cell lines to create QC materials that mimic real patient samples, however this approach is technically more difficult.

- Synthetic nucleic acids are easy to prepare in large quantities using PCR and subcloning methods. Synthetic control materials may be used for controlling multiplex assays and may be cost effective, with the ability to accommodate rapidly changing technology used to perform the tests. The major disadvantage of using synthetic nucleic acids as control materials is the difference between them and actual patient specimens.
- Standardized quantitative RT-PCR assays using multiple internal standards provide the ability to measure gene expression and compare results generated by researchers in different laboratories or institutions. However, this approach is more suitable for academic and pharmaceutical research rather than clinical genetic testing.
- Dried blood spots (DBS) provide a stable matrix for DNA and could be considered for PT and EQA challenges. In addition, DBS provide the ability to monitor specimen extraction process and therefore may be considered a better control material compared to purified DNA. However laboratories performing genetic testing may not accept such challenges if they have not validated the testing procedures using dried blood spots as a specimen source.

*Areas of Need for QC Materials*

Participants identified the following as the most important areas of need for QC materials:

- Genetic tests that are routinely offered in clinical and public health practice.
- Molecular infectious disease testing and molecular hematology/oncology testing are two new areas that need QC materials.
- Control materials are needed for monitoring multiple steps of the analytic process. For example, controls allowing monitoring of both nucleic acid extraction and amplification would be needed for RT-PCR assays for leukemia testing.
- Appropriate QC materials are needed for test validation. Comments were made that laboratories developing tests based on published literature should not assume that sufficient assay validation had been performed by the authors; similarly, laboratories should verify control materials provided by manufacturers along with the test kits, even if the test system has been cleared or approved by FDA.
- Negative controls would be also needed in addition to positive control materials.
- Appropriate control materials for sequencing assays need to be further discussed.
- Control materials representing genetic diversity among different race/ethnic groups are needed for detecting population-associated polymorphisms that routine QC materials may not be able to monitor. The race/ethnicity panels from Corriell were recommended as a source of control materials to meet such needs. It was also pointed out that self-reporting was not reliable for determining race/ethnicity origins, and that genotyping might provide better classification for genetic backgrounds in the future.

Participants felt that it would be relatively easy to address areas such as single gene assays, testing for single SNPs or single mutations, and high-volume or commercialized testing for which the costs of developing and validating QC materials could be shared by laboratories offering the tests. In contrast, array assays and panel tests, such as CF testing, are among those for which control materials are more difficult to develop. Participants recommended that a

priority list be developed for areas needing QC materials, focusing on tests that are currently offered in clinical and public health practice, particularly those considered as routine or high-volume testing. It was also recommended that control materials that already have been generated and validated, such as those developed by the CDC-funded projects, be made more widely available.

*Impact of Patents and Licensing Agreements*
Regarding impact of patents and licensing agreements on the development and distribution of QC materials, concern was expressed that laboratories would incur increased costs by paying royalty or licensing fees for the control materials while reimbursement for performing the tests remained unchanged. The impact of patents or licensing agreements on control materials as a component of test kits also would likely lead to increased kit costs. It was suggested that the impact of gene patents and technology patents, and exclusive licenses and non-exclusive licenses be considered separately. If patents or licensing agreements do not restrict the use of specific technology for QC material development, they should not prevent laboratories from making control materials in house. Panelists commented that patenting and licensing are common to both industry and academic institutions seeking to protect their intellectual property; as a measure to help ensure access to results of federally funded research, NIH could require its grantees to establish only non-exclusive licensing agreements in seeking commercialization of their research products. Participants agreed that issues related to patenting and licensing should be considered in view of the global marketplace and should not be underestimated.

Panel Discussion of Practical Issues

The discussion was moderated by Dr. Wayne Grody. Panelists included Dr. Jean Amos, Dr. David Barton, Dr. Linda Bradley, Dr. David Ledbetter, Dr. Tom Prior, and Dr. Walter Noll.

*Programmatic Needs for Development and Provision of QC Materials*
Participants agreed that there is a great need for a structured organization to coordinate activities of stakeholders and identify funding venues. A translational research model is also needed for transferring tests from research phase to clinical use, particularly for rare diseases.

*Informed Consent*
Concern was raised on obtaining informed consent for using residual patient specimens for QC/QA purposes after completion of clinical testing and reporting of test results, particularly in light of the international proposals to allow patients to request laboratories to discard residual specimens as soon as patient testing is completed. Participants agreed that an acceptable process would be needed for using residual patient specimens for QC/QA purposes. It was suggested that the CLIAC recommendations regarding retention and uses of residual patient specimens be explored as potential approaches. It was agreed that the informed consent issues are very important and the roles of three federal regulations – the Common Rule, 21 CFR regarding the FDA role, and the HIPAA Privacy Rule would need to be considered. Participants also recommended working with the Advisory Committee to the Office of Human Research Protection and the Secretary's Advisory Committee on Genetics, Health, and Society to address the informed consent issues.

*Validation of QC Materials*

Participants were in agreement that they would not purchase QC materials that had not been validated. The following suggestions were made regarding mechanisms for validating QC materials:

- The College of American Pathologists (CAP)/American College of Medical Genetics (ACMG) molecular pathology program could provide a mechanism for QC material validation. However, concerns were raised that CAP might not be willing to use unvalidated materials in genetic testing surveys since they could lead to ungraded challenges; in addition, there might not be enough laboratories to achieve statistical power. Therefore, the process would need to be improved for laboratory enrollment and performance assessment before considering the CAP/ACMG programs for this effort.
- The Association for Molecular Pathologists (AMP) was suggested as another mechanism for recruiting laboratories to participate in efforts to validate QC materials.
- Test developers and diagnostic manufacturers can contribute by validating control materials for the tests they develop.
- A cell exchange network might be formed of laboratories listed in the GeneTests directory to validate control materials.
- Genetic Alliance could help to a certain extent in establishing a biobank of patient specimens to facilitate genetic research studies.

*FDA Oversight for Control Materials*

Dr. Maria Chan clarified that most control materials are considered as Class I biologicals under 510(k) or 513(g) by FDA. Although the FDA guidances on QC materials are not specific for genetic tests, they should be useful as a reference for evaluating QC materials for genetic tests. It was noted that vendors of Analyte Specific Reagents (ASRs) were reluctant to provide control materials together with the ASRs because doing so might violate the ASR rule. One participant commented that several manufacturers had proposed to FDA an "in vitro analytical test" model, which would require only analytical claims and leave the demonstration of clinical utility to the laboratories. However, concern was expressed about simply stating which alleles were detected in test reports. Dr. Chan emphasized the strength of 510(k) for patient testing by requiring submission of relevant clinical information. It was also noted that test validation would be more straightforward if there were more FDA approved tests.

*Appropriate Types of Control Materials*

Several participants commented that cell lines would be preferred to purified DNA by providing the capability of monitoring the extraction process; but the choice of control materials should also depend on the application. It was suggested that artificial constructs, which could circumvent the process of obtaining informed consent for using residual specimens, might be used as QC materials for some applications. Participants agreed that it would be important to examine the entire analytical process to determine appropriate control materials for the testing.

*Rare Disease Testing*

Participants agreed that the quality of rare disease testing would need to be improved. Comments were made that it might not be possible to have controls for all rare diseases; and because many families with rare diseases carry private mutations, testing would need to be

performed by sequencing methodology. The following suggestions were made to address the needs in rare disease testing:

- In light of the limited number of referral laboratories for rare diseases, a national network similar to the European networks should be established.
- The biobank being developed by Genetic Alliance may be helpful in the long term in collecting specimens containing private mutations.
- Control materials are needed for tests that evaluate targeted mutations.
- Although sequencing has built-in internal controls, positive and negative materials are needed to validate sequencing procedures for rare disease testing.

*Costs Concerns*
Participants agreed that costs of control materials should not be prohibitive since laboratories already are paying high costs for testing reagents and royalties. A charge of $50 would be acceptable for a DNA pellet that could provide QC materials for several tests.

Panel Discussion on Bridging the Gaps and Sustaining a Process for QC Materials

This panel discussion was moderated by Dr. Daniel Farkas. Panelists included Dr. Jean Beck, Dr. Maria Chan, Dr. Catherine O'Connell, Dr. Tim O'Leary, and Dr. Beth Rohlfs.

*Approach to Development, Validation, Collection and Storage, and Distribution of QC Materials*
Dr. Jean Beck provided a possible scenario to meet the needs for cell lines to be used as QC materials for inherited genetic disorders, including submission process, validation schemes, and distribution procedures. She clarified that Corriell is ready to accept residual specimens and can provide packaging for submission. It was suggested that the AMP listserv and website could be used to inform the laboratory community about these capabilities. Laboratories could also help Corriell to validate cell lines as QC materials. It was further recognized that an organized structure would be needed to support and coordinate this process.

*Priorities for QC Material Development*
Participants proposed that a priority list be established for developing QC materials. This could be accomplished by working with CAP and ACMG to determine the needs for provision of challenges in the CAP/ACMG genetics surveys, and by surveying member laboratories of a professional organization such as AMP. One of the priorities identified was mutations that had not been included as challenges in the CAP/ACMG surveys.

*Role of Government in Helping Sustain the Process*
It was suggested that the NIST certificate could be a mechanism for provision of quality QC materials. Dr. O'Connell commented that the role of NIST, which is under the Department of Commerce instead of HHS, should be clarified for the laboratory community. Participants suggested that efforts be made to coordinate activities of federal agencies and help determine the level of funding needed to sustain the processes for QC materials development. It was recognized that the government could have a major role in developing appropriate QC materials for genetic testing.

In addition, several participants credited the QC rule in requiring laboratories to have control procedures to monitor the accuracy and precision of the complete analytical process; however, a practical gap was pointed out that there are no guidelines currently for CMS to evaluate test validation and to ensure the use of appropriate controls.

**Next Steps**

At the conclusion of the meeting, participants agreed that QC materials could be obtained and made available at a reasonable cost. The participants identified the following eight areas that should be addressed for developing and providing QC materials for genetic tests:

1) Raise awareness about research activities on developing QC materials and facilitate collaboration among research efforts;
2) Develop better coordination of funding sources and opportunities;
3) Develop professional guidance on appropriate QC practices;
4) Clarify regulatory requirements for providers of QC materials;
5) Develop validation processes for QC materials;
6) Develop processes to use existing cell banks as sources for QC materials;
7) Develop a scheme to set priorities for QC materials; and
8) Develop networks of contributors of QC materials.

Participants acknowledged the broad scope of these issues and recognized the need to focus effort toward each of them. Therefore, a total of eight workgroups were assigned, each attempting to address one of the identified needs. The workgroup leaders will form a steering committee to coordinate activities among the workgroups. The next meeting is proposed for March 8, 2004, in Orlando, Florida, to review workgroup progress and move forward with a plan of action.